Determination of the Bioavailability of Novel Antisense Chemistries Using Analogs of a Phosphorothioate Oligonucleotide in Mice

ISIS 7818 C·A·G·C·C·A·T·G·G·T·T·C·C·C·C·C·C·A·A·C
(2'-OMe)

ISIS 7817 C·A·G·C·C·A·T·G·G·T·T·C·C·C·C·C·C·A·A·C
(2'-OPropyl)

ISIS 13251 C·A·G·C·C·A·T·G·G·T·T·C·C·C·C·C·C·A·A·C
(2'-MOE)

Key: A, C, G, T = 2' deoxyribose
A, C, G, T = 2' O-modified ribose
· = phosphorothioate

FIGURE 1

Effect of 2'- Modifications of Oligonucleotides on Oral Bioavailability[1] in Mice

Oligonucleotide	I.V. Systemic Tissue[a] (% Dose)	Oral Systemic Tissue[a] (% Dose)	% Bioavailability[b]
7818 (2'-OMe)	18	0.35	1.9
7817 (2'-OPropyl)	22	0.31	1.4
13251 (2'-MOE)	7	1.46	20.9[c]

a liver + kidney
b ral/I.v. x 100
c may overestimate actual bioavailability due to observed intestinal degradation of MOE compounds
1 Radiolabel ^{35}S is associated with 5' end; Total radioactivity is measured

FIGURE 2

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Bioavailability Analysis of Backbone Modified Oligonucleotide Using Analogs of the Phosphorothioate Oligonucleotide ISIS 4189

ISIS 4189 CsAsGsCsCsAsTsGsGsTsTsCsCsCsCsCsCsAsAsC

ISIS 14182 CmAoGmCoCmAsTsGsGsTsTsCsCsCsCmCoCmAoAmC
3/3 MMI

ISIS 14183 CmAoGmCoCmAsTmGoGmToTmCoCmCoCmCoCmAoAmC
Fully alternating PO/MMI

Key: s, P=S linkage; o, P=O linkage; m, MMI linkage

FIGURE 3

MMI- Modifications Appear to Improve Oral Bioavailability of Oligonucleotides in Mice

Oligonucleotide	I.V. Systemic Tissue[a] (%Dose)	Oral Systemic Tissue[a] (%Dose)	% Bioavailability[b]
14182 MMI (3/3 P=O in the wings)	16.5	1.9	11.5
14283 MMI (alternating P=O throughout)	10.0	1.6	16.0c

a (carcass- (skin+skeletal muscle)); 8 hours post dose
b oral/i.v. x 100
c may be overestimated due to rapid tissue clearance of this compound

FIGURE 4

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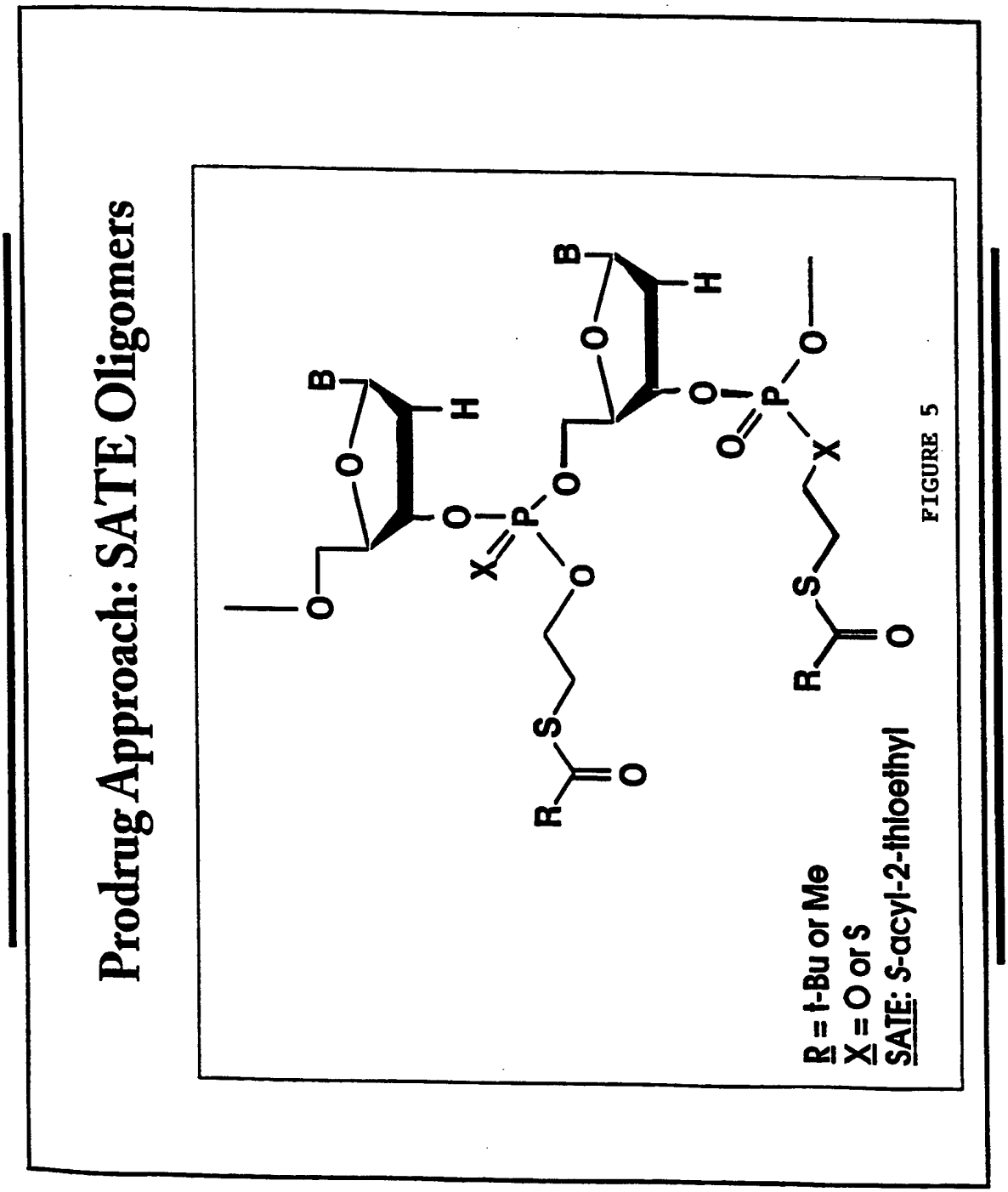

FIGURE 5

Prodrug Modifications Appear to Improve the Oral Bioavailability of Phosphorothioate Oligonucleotides in Mice

Oligonucleotide	I.V. Systemic Tissue[a] (%Dose)	Oral Systemic Tissue[b] (%Dose)	% Bioavailability[c]
t-butyl SATE (T_{12} P=S)	42	10-18	9-16
t-butyl SATE (T_{12} P=O)	81	10-13	2.6-3.4

a (liver + kidney) + (carcass); 1 hour post dose; total radioactivity
b (liver + kidney) + (carcass); 1-4 hours post dose; total radioactivity
c (oral/I.v. total radioactivity x oral/I.v. oligo associated radioactivity) x 100

FIGURE 6

Intestinal Absorption of Methoxyethoxy Derivatives of ISIS 5132 / CGP 69846A Evaluated in Rat Model

ISIS 5132 CGP 69846A	TsCsCsCsGsCsTsGsTsGsAsCsAsTsGsCsAsTsT
ISIS 13650 CG# 1740 CGP 71849A	TsCsCsCsGsCsTsGsTsGsAsCsAsTsGsCsAsTsT
ISIS 12854 CG# 1739 CGP 69845A	ToCoCoCoGoCsTsGsTsGsAsCsAsToGoCoAoToT

Key:
A, C, G, T = 2' deoxyribose
A, C, G, T = 2' MOE modified ribose
o = phosphodiester
s = phosphorothioate

FIGURE 7

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Rate of Absorption of Oligonucleotides in Different Sites of the Rat Intestine

Compound	Site	Absorption Rate[1] (µg/min*cm)
5132	Duo/Jej	nd
	Ileum	0.2
	Colon	0.2
13650[a]	Duo/Jej	0.1
	Jejunal	0.8
	Ileum	0.7
	Colon	0.9
12854[a]	Duo/Jej	0.9[2]
	Duo/Jej	0.1
	Ileum	1.8
	Colon	1.7

a Internally labeled with ^{35}S

1 Absorption Rate = (amount of drug lost - amount bound to tissue/mucosa)/(time*length of intestinal segment): COMPARE With THEOPHYLLINE = 9 µg/min*cm

2 Bile present; May be a confounding variable

nd not detectable

FIGURE 8